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Influence of Solvents on the Variety of Crystalline Forms of Erythromycin

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Sabiruddin Mirza[1], Inna Miroshnyk[1], Jyrki Heinämäki[1], Leena Christiansen[1], Milja Karjalainen[1], and Jouko Yliruusi[1,2]

[1]Pharmaceutical Technology Division, Department of Pharmacy, University of Helsinki, Finland
[2]Viikki Drug Discovery Technology Center, University of Helsinki, Finland

ABSTRACT

The influence of the organic solvents widely used in the pharmaceutical industry (acetone, methylethylketone, ethanol, and isopropanol) both in the presence and in the absence of water on the crystallization behavior of erythromycin (Em), a clinically relevant antibiotic of the macrolide group, was investigated. It was observed that despite a high preference for water as a guest molecule, Em rather easily forms solvates with the organic solvents studied. Consequently, 4 distinct solvates of Em have been isolated by recrystallization: acetonate, methylethylketonate, ethanolate, and isopropanolate. It was established that in a pure organic solvent, or 1:9 or 1:1 water–organic solvent mixtures, the corresponding solvate is always crystallized. However, the recrystallization of erythromycin from 2:1 water–organic solvent (excluding methylethylketone) mixture results in the formation of a crystal hydrate form. X-ray powder diffraction revealed the isostructurality of the solvates with acetone and methylethylketone. Thermogravimetric analysis showed that the loss of volatiles by all of the solvated crystals is nonstoichiometric. The desolvation behavior of the solvates with the organic solvents studied by means of variable-temperature x-ray powder diffraction indicates that in contrast to erythromycin dihydrate, they belong to a different class of solvates—those that produce an amorphous material upon desolvation.

KEYWORDS: erythromycin, crystallization, solvated crystals, isostructurality, clathrate, x-ray diffraction

Corresponding Author: Sabiruddin Mirza, Pharmaceutical Technology Division, Department of Pharmacy, University of Helsinki, Finland; Tel: 358 9 19159151; Fax: + 358 9 19159144; Email: sabir.mirza@helsinki.fi.

INTRODUCTION

The solid-state physical properties of a drug are generally influenced by the existence of different crystalline and amorphous forms, some of them polymorphs, others pseudopolymorphs (solvated crystalline forms). This opens up a challenging avenue for the investigation of drug polymorphism and formation of various solvates. The properties of different forms of a drug (crystal structure, habit and degree of crystallinity) may influence the manufacturing process, dissolution rate, storage stability, and bioavailability of the drug product.[1-3]

Erythromycin dihydrate ($Em \cdot 2H_2O$) is an important antibiotic of the macrolide group that has been in clinical use for over 50 years. Em base exists in different forms: anhydrate, monohydrate, dihydrate, and various solvates, and each of these forms can be crystalline, partially crystalline, or amorphous. A number of studies on these forms have been reported.[4-11] At the same time, a literature review showed that similar information on commercially marketed lots of Em raw material is often lacking, and significant variations in the physical properties of the bulk drug are encountered among material supplied by some manufacturers and among different lots supplied by the same manufacturer.[6] Additionally, the intricate molecular structure of Em (**Figure 1**) suggests the variety of possible supramolecular motifs in crystals, resulting in the formation of different polymorphic modifications. Therefore, the development of methods of control for the crystallization process of Em in order to produce a drug possessing favorable physical, technological, and pharmacokinetic properties is of great practical interest.

The purpose of the present study was to investigate the variety of crystalline forms that are obtained in the crystallization of Em from different organic solvents both in the presence and in the absence of water. X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and optical microscopy were used to identify and evaluate physical and structural properties of the resulting crystalline phases.

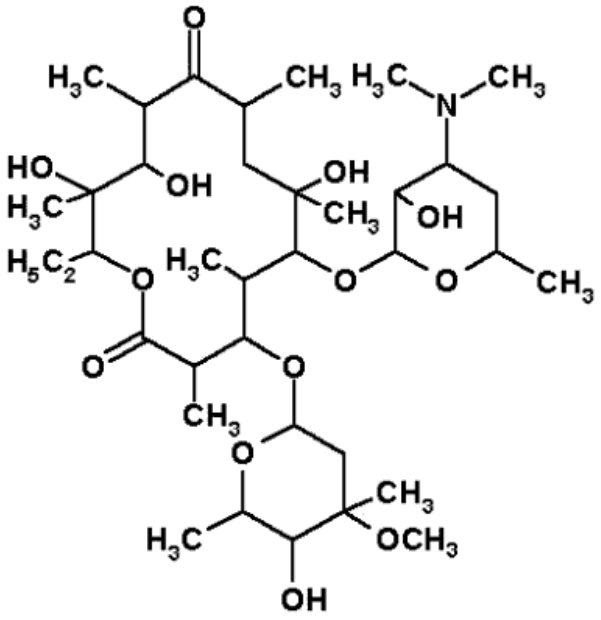

Figure 1. Structural formula of erythromycin.

MATERIALS AND METHODS

Materials

Em (99.6% pure) was commercially available (Pharmacia & Upjohn Company, Kalamazoo, MI). Purified distilled water (Ph Eur) and the following analytical-grade organic solvents were used in the crystallization experiments: ethanol (EtOH), isopropanol (*i*-PrOH), acetone (Ac), and methylethylketone (MeEtCO).

Recrystallization of Em

Screening crystallization of Em with different solvents was carried out using an automated 24-well reaction block crystallizer (Variomag 24.4-1, H+P Labortechnik GmbH, Munich, Germany). Cooling, heating, and stirring rates were measured and controlled by a CC computer program (Huber, Offenburg, Germany). Em·$2H_2O$ was recrystallized from solutions in the above-mentioned solvents or in 1:9, 2:1, and 5:5 (vol/vol) water–organic solvent mixtures. The crystals harvested were filtered through a Büchner funnel (Haldenwanger Technische Keramik GmbH, Berlin, Germany), dried in a vacuum oven at 40°C for 12 hours, and stored in a desiccator at room temperature (21 ± 1°C). The ethanolate crystals were kept in the mother liquor.

XRPD

XRPD studies were made using a theta-theta diffractometer (D8 Advance, Bruker axs GmbH, Karlsruhe, Germany). The XRPD experiments were performed in a symmetrical reflection mode using CuKα radiation (1.54 Å) at 40 mA and 40 kV using Göbel Mirror bent gradient multilayer optics (Bruker axs GmbH, Karlsruhe, Germany). The scattered intensities were measured with a scintillation counter. The angular range was from 4° to 50° with steps of 0.05°, and the measuring time was 1 second/step. The samples were measured at different temperatures using variable-temperature XRPD. The instrumental broadening estimated from the reflection of silicon was 0.03°. The crystallinity was estimated by fitting a linear combination of the intensities of the crystalline and amorphous component to the diffraction patterns of the samples. The intensity curve of the sample, which had been changed to being totally amorphous, was used as the amorphous background, and the intensity curve where the amorphous background was subtracted was used as the crystalline model intensity curve. The crystallinities of the samples were calculated as the ratio of the integrals of the intensities of the crystalline part and the sample studied.

DSC

A DuPont differential scanning calorimeter (Model 910S, TA Instruments, New Castle, DE) equipped with a data station (Thermal analyst 2000, TA Instruments, New Castle, DE) was used to determine the DSC curves representing the rates of heat uptake with respect to temperature. The temperature axis and the cell constant of the DSC cell were calibrated with indium (10 mg, 99.999% pure, melting point 156.6°C, heat of fusion 28.4 J/g). The solid sample (3-4 mg) was weighed into an aluminum pan, and at a heating rate of 10°C/min DSC studies were carried out in open pans under static air.

TGA

TGA of the crystalline samples was performed with a Mettler TGA analyzer (model 850, Mettler-Toledo, Greifensee, Switzerland). Samples (10 mg) were analyzed in open aluminum pans under a nitrogen flow (50 mL/min) at 25 to 200°C with a heating rate of 10°C/min. The temperature scale of the equipment was calibrated with zinc and indium, while the microbalance was calibrated with calcium carbonate.

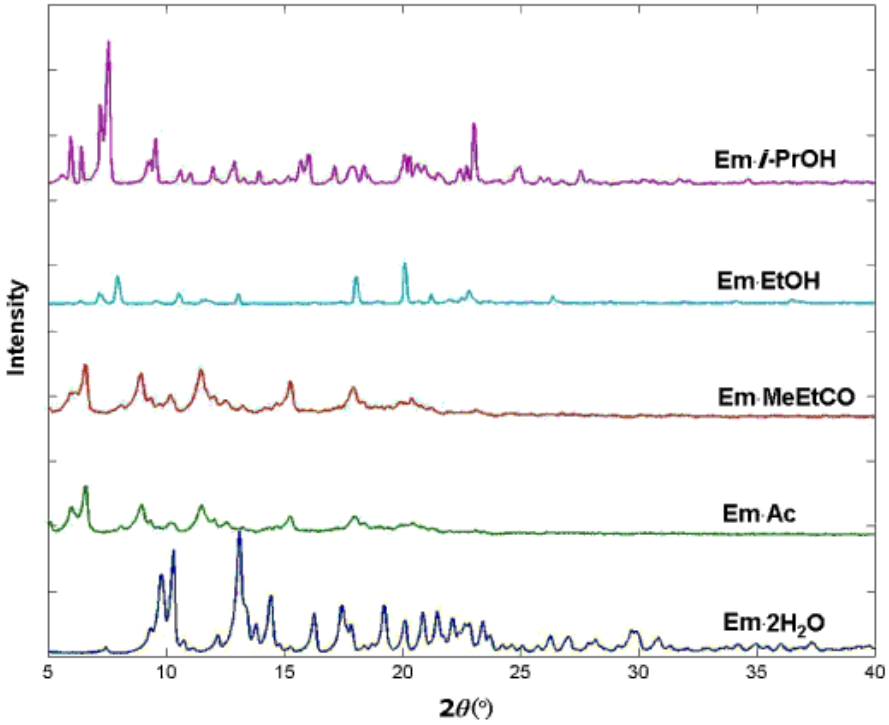

Figure 2. XRPD patterns of different crystalline products of Em: Em·2H_2O, Em·Ac, Em·MeEtCO, Em·EtOH, Em·*i* -PrOH.

Optical Microscopy

To reveal the morphological differences between the crystals, microscopic observations on samples suspended in cedar wood oil (Riedel-de Haën, GmbH, Seelze, Germany) were performed using an optical microscope (DAS Mikroskop Leica Microscopie und Systeme GmbH, Wetzlar, Germany) equipped with a videocamera.

RESULTS AND DISCUSSION

XRPD

It was observed that Em rather readily forms solvates with the organic solvents studied; consequently, besides Em·2H_2O, 4 crystalline products—acetonate (Em·Ac), methylethylketonate (Em·MeEtCO), ethanolate (Em·EtOH), and isopropanolate (Em·*i*-PrOH)—were found and identified by XRPD. It appeared that the recrystallization from a pure organic solvent as well as from 1:9 and 1:1 water–organic solvent mixtures leads to a corresponding solvate. For 2:1 water–organic solvent mixture, a dihydrate was always formed, with the exception of the water–MeEtCO recrystallization system, from which only crystals solvated with MeEtCO were obtained.

The XRPD patterns of the crystals Em·Ac, Em·MeEtCO, Em·EtOH, and Em·*i*-PrOH differ substantially from that of Em·2H_2O (**Figure 2**). The diffractograms of Em·EtOH and Em·*i*-PrOH are also readily distinguishable from those of Em·Ac and Em·MeEtCO and from one another. The diffraction patterns of Em·Ac and Em·MeEtCO, on the other hand, are very similar. Superposition of these confirms that the position of the majority of the peaks is in virtual correspondence, leading to the conclusion that these 2 phases are isostructural.[12] The quantitative characteristics of the main reflections of the diffraction patterns of Em·2H_2O and Em·Ac, Em·MeEtCO, Em·EtOH, and Em·*i*-PrOH (angle 2θ and relative intensity I) are given in **Table 1**.

The crystal structure of Em·2H_2O is well studied.[8,13] It is a clathrate where large molecules of Em play the role of "hosts" and small molecules of water play the role of "guests" occupying periodic voids in the crystal. It was hypothesized that solvates obtained in the present study have an analogous—that is, a clathrate—structure. However, in these cases, the role of guests is performed by small organic molecules. In this context, the isostructurality of Em·Ac and Em·MeEtCO is easily explainable. Inclusion of the structurally close guest molecules Ac or MeEtCO in the crystal lattice does not result in the formation of 2 different crystal structures but leads to the creation of the same 3-dimensional hosting framework.

Table 1. X-ray Powder Diffraction Data*

Em·2H_2O		Em·Ac		Em·MeEtCO		Em·EtOH		Em·*i*-PrOH	
2θ,°	**I, %**	**2θ,°**	**I, %**	**2θ,°**	**I, %**	**2θ,°**	**I, %**	**2θ,°**	**I, %**
9.79	65	5.97	61	5.99	52	7.18	29	5.99	35
10.26	85	6.59	100	6.58	100	7.94	67	6.43	28
13.07	100	8.94	64	8.92	85	10.54	27	7.25	57
13.40	40	9.29	35	9.30	42	13.02	26	7.55	100
14.43	49	11.48	65	10.19	47	18.02	66	9.52	34
16.26	35	12.04	38	11.49	91	20.09	100	16.04	23
17.42	41	12.57	32	12.02	46	21.25	24	20.04	22
17.83	25	15.21	43	12.49	37	22.83	33	20.26	21
19.24	40	17.97	43	15.25	71			23.05	44
20.86	36	18.38	30	17.92	61				
				20.37	41				

*Em indicates erythromycin; Ac, acetone; MeEtCO, methylethylketone; EtOH, ethanol; *i*-PrOH, isopropanol.

DSC

DSC curves of different crystalline products of Em are depicted in **Figure 3**. Both the onset temperatures and enthalpies of desolvation were considered to reveal the structural features by means of thermal analysis. As can be seen in **Figure 3**, Em·2H_2O exhibits 2 endothermic transitions. There is the first broad peak with the onset temperature of 50.7 ± 1.9°C and an enthalpy change of 157 ± 3 Jg^{-1} (n = 3) attributable to the dehydration process. The second endothermic effect, at 124.4 ± 3.2°C, is due to melting (7 ± 0.2 Jg^{-1}, n = 3). The onsets of desolvation for the crystals Em·Ac and Em·MeEtCO are observed at temperatures near the boiling points of their respective solvents (56.5 ± 1.4°C, 59 ± 1 Jg^{-1}, and 72.3 ± 1.9°C, 68 ± 1 Jg^{-1} [n = 3], respectively). Both broad endothermic peaks at 54 ± 1.3°C and 75 ± 2.6°C (68 ± 1 and 29 ± 1 Jg^{-1}, respectively) observed in the DSC profile of Em·EtOH correspond to desolvation, which was detected by TGA. For Em·*i*-PrOH, there is a rather sharp endotherm at 90.8 ± 1.2°C with an enthalpy change of 143 ± 5 Jg^{-1} (n = 3) due to desolvation, as has been confirmed by TGA.

Overall, depending on the desolvation onset temperature, the solvates of Em may be divided into 2 groups with different strengths of host–guest interactions. The first group includes Em·Ac, Em·MeEtCO, and Em·*i*-PrOH, for which the onset temperatures of desolvation are practically coincident with the normal boiling points of the pure solvents. The guest releasing for the solvates of the second group (Em·2H_2O and Em·EtOH) starts at lower temperatures. Additionally, the relative sharpness of the DSC endotherms for the desolvation of Em·Ac, Em·MeEtCO, and Em·*i*-PrOH may be attributed to the location of their guest molecules in isolated sites in the crystal.[14] In contrast, the desolvations of Em·2H_2O and Em·EtOH are reflected as broad endotherms, suggesting a different topology of solvent inclusion (apparently, channel type).

TGA

Studies of thermal decomposition behavior using TGA were performed to draw conclusions concerning the existence and stoichiometry of different phases for the host–guest systems under investigation (**Table 2**). TGA results showed that the guest release occurs in 1 step in all solvates of Em except Em·EtOH (**Figure 4**). The loss of 2 water molecules in Em·2H_2O takes place between 40 and 100°C. Both the low desolvation onset temperature and 1-step process are in good agreement with the topology of Em as a channel-type hydrate.[13] For Em·Ac and Em·MeEtCO, the guest release occurs in the temperature interval 50 to 70°C and 70 to 90°C, respectively. Em·*i*-

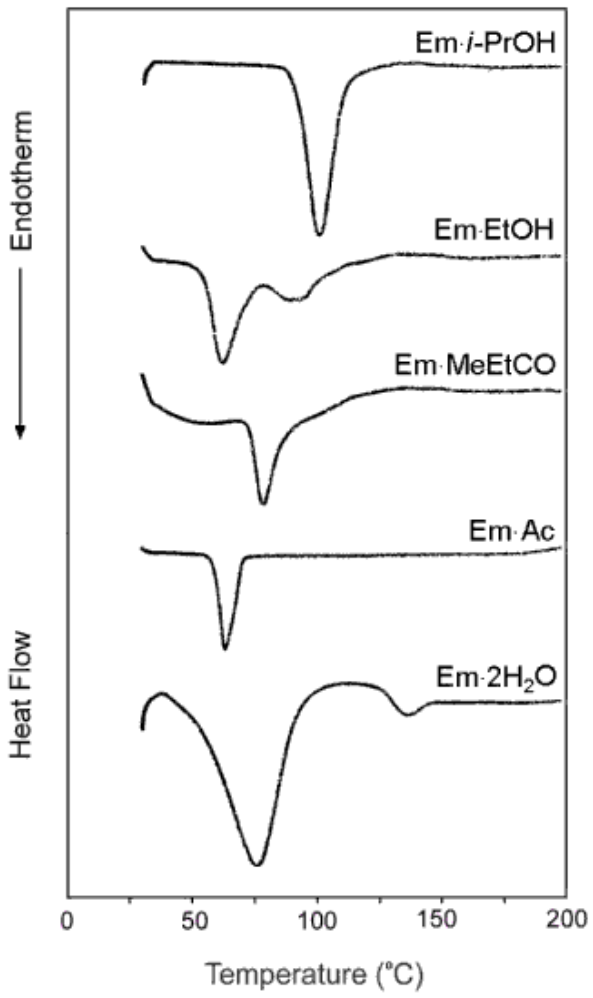

Figure 3. DSC curves of Em host–guest systems: Em·2H_2O, Em·Ac, Em·MeEtCO, Em·EtOH, Em·*i* -PrOH.

PrOH decomposes starting at 90 and finishing at 130°C. No indication for the formation of any intermediate phase in the TGA traces for Em·Ac, Em·MeEtCO, and Em·*i*-PrOH was found. In contrast, the desolvation of Em·EtOH occurs in 2 steps; this was also observed in the DSC curve, indicating the existence of an intermediate phase. It gives rise to the assumption that the guest molecules have different binding strengths and, therefore, nonequivalent environments within the crystal lattice. The first step takes place between 40 and 75°C and results in the loss of 2 guest molecules that have lower interaction energy and, evidently, are localized in solvent tunnels. The second step, in the temperature range of 75 to 110°C, releases the remaining solvent molecule and leads to the guest-free host formation. Overall, TGA showed that the loss of volatiles by the solvated crystals is nonstoichiometric, so that **Table 2** presents the maximum host–guest molar ratios.

Variable-temperature XRPD

In addition to DSC and TGA, variable-temperature XRPD was performed to identify and evaluate the phases formed during the desolvation process of the host–guest systems of Em. The diffraction patterns registered for this purpose are shown in **Figure 5**. The crystal structure of Em·2H_2O does not change up to 50°C (**Figure 5A**). Above 50°C, corresponding to the dehydration onset temperature of Em·2H_2O, some peaks on the diffractogram disappear and new ones appear, although the basic features of the XRPD pattern remain constant. Specifically, this means that dehydration of Em·2H_2O does not destroy its original crystal lattice but results in the formation of the isostructural guest-free host. The latter remains crystalline up to 120°C. At 130°C, as a result of the melting, a significant weakening of the diffraction maximums is observed, and at 140°C the substance becomes completely amorphous. It is noteworthy that at 25°C, the degree of crystallinity of Em·2H_2O is 89%. During heating, however, a slight increase (40-70°C, 91%) is followed by a decrease (80°, 82%; 90-120°, 74%; 130°, 20%; 140°, 0%).

Em·Ac and Em·MeEtCO behave similarly during the thermal decomposition. However, the mechanism and kinetics of desolvation of those differ from that of Em·2H_2O, as was suggested from their DSC traces. As can be seen in **Figure 5B**, the XRPD pattern of Em·MeEtCO remains practically invariable up to 80°C, when the releasing of the guest molecules occurs. Increasing the temperature further results in the formation of an amorphous phase that was confirmed by DSC. This leads to the conclusion that the loss of the solvated molecules results in the collapse of the host crystal lattice.

The desolvation of Em·EtOH resulting in the formation of the crystals with an opaque appearance was observed under ambient conditions. This phenomenon may be attributed to the location of the guest molecules in the intersecting solvent tunnels running within the crystal lattice[15] that was also deduced from the DSC trace. The complete loss of the solvent of crystallization leads to the formation of amorphous material.

Unlike the other host–guest complexes of Em, for Em·*i*-PrOH, no change of XRPD pattern, except some increasing crystallinity, was observed below 90°C (**Figure 5C**); this corresponds to its greater stability to desolvation. The diffractogram registered at 100°C, which corresponds to the partially desolvated host, however, does not differ distinctly from that of the solvate but rather repre-

Table 2. Composition of the Host–Guest Systems of Em Determined by TGA*

Guest Molecule	TGA Weight Loss, %	Maximum Host:Guest Molar Ratio
H_2O	4.59	1:2
Ac	7.59	1:1
MeEtCO	11.61	1:1
EtOH	18.96	1:3
i-PrOH	16.04	1:2

*TGA indicates thermogravimetric analysis; Ac, acetone; MeEtCO, methylethylketone; EtOH, ethanol; *i*-PrOH, isopropanol.

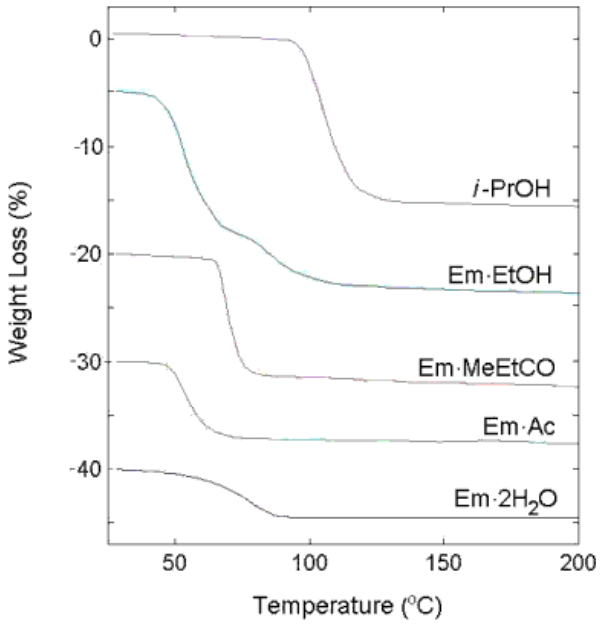

Figure 4. TGA traces of Em host–guest systems: Em·2H_2O, Em·Ac, Em·MeEtCO, Em·EtOH, Em·*i* -PrOH.

sents the same profile of lower crystallinity. Above 110°C the XRPD pattern continuously lost structure, suggesting an increasingly amorphous character (the crystallinity of the sample is 80%, 83%, 39%, and 0% at 25, 70, 100, and 120°C, respectively).

Optical Microscopy

It was observed that in the presence of water Em forms platelike crystals of Em·2H_2O (**Figure 6A**), whereas the crystals of Em·Ac, Em·MeEtCO (**Figure 6B**), and Em·*i*-PrOH (**Figure 6C**) have a thin needle shape. The exception is Em·EtOH (**Figure 6D**), whose plate-like crystals look like those of Em·2H_2O but can be observed easily without magnification.

CONCLUSION

The influence of the organic solvents widely used in the pharmaceutical industry (Ac, MeEtCO, EtOH, and *i*-PrOH) both in the presence and in the absence of water on the crystallization behavior of Em was investigated. It was established that despite a high preference for water as a guest molecule, Em rather easily forms solvates (namely, Em·Ac, Em·MeEtCO, Em·EtOH, and Em·*i*-PrOH) with the organic solvents studied. More precisely, in a pure organic solvent, or 1:9 or 1:1 water–organic solvent mixtures, the corresponding solvate is always crystallized. Excluding MeEtCO, the recrystallization of Em from 2:1 water–organic solvent mixtures favors a crystal hydrate form. XRPD revealed the isostructurality of the solvates with Ac and MeEtCO that may be attributable to the similar structure and the same hydrogen bonding capacity of those guest molecules. Though it was initially believed that all the solvates obtained have structures similar to the dihydrate—that is, a clathrate structure—no experimental evidence was found to support such a hypothesis. None of the solvates studied exhibits stoichiometry or forms isostructural desolvates like Em·2H_2O. Instead, the desolvation of the solvates results in collapse of the crystal lattice and formation of an amorphous material.

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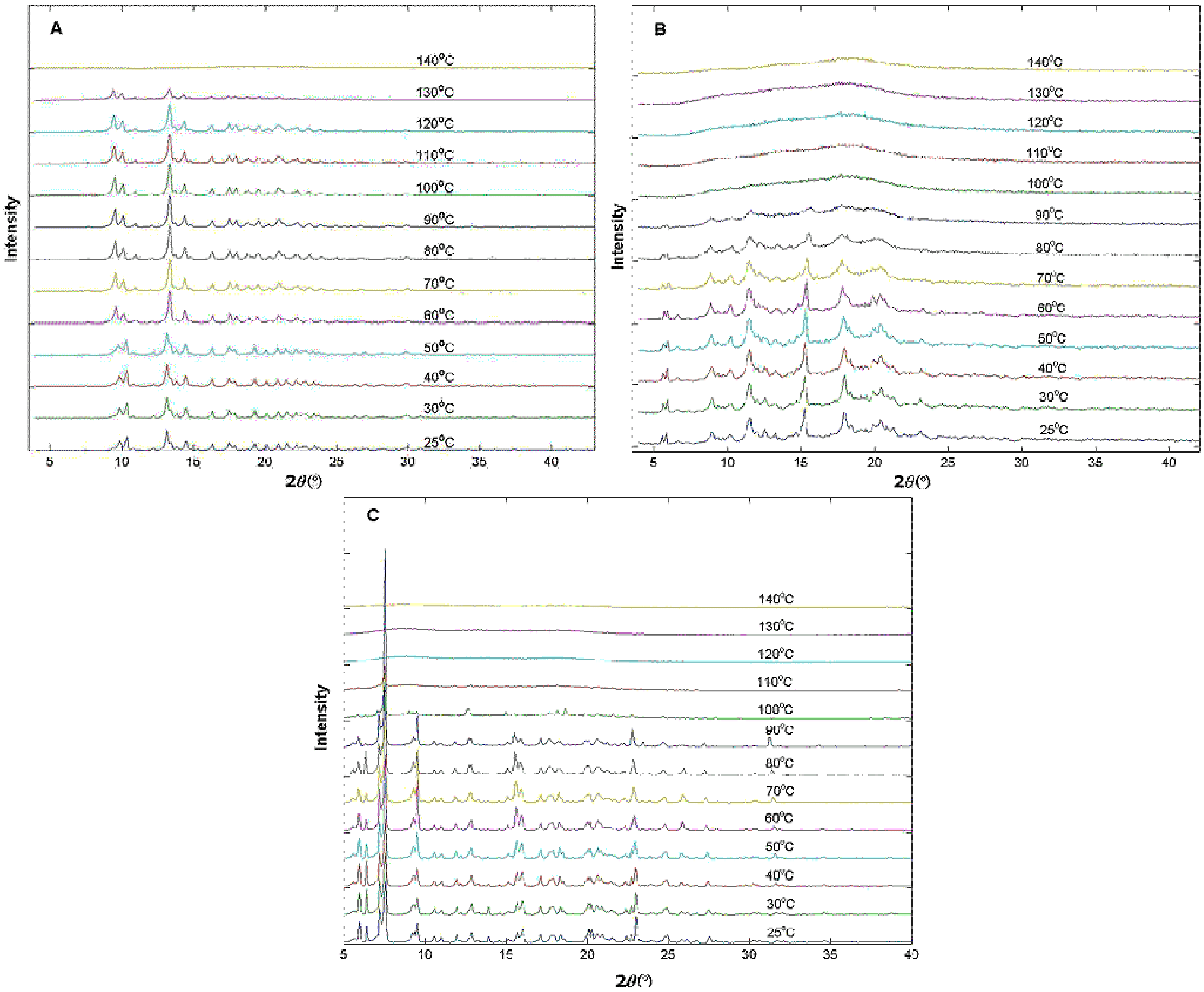

Figure 5. Temperature-dependent XRPD patterns: (A) Em•2H_2O, (B) Em•MeEtCO, (C) Em•*i* -PrOH.

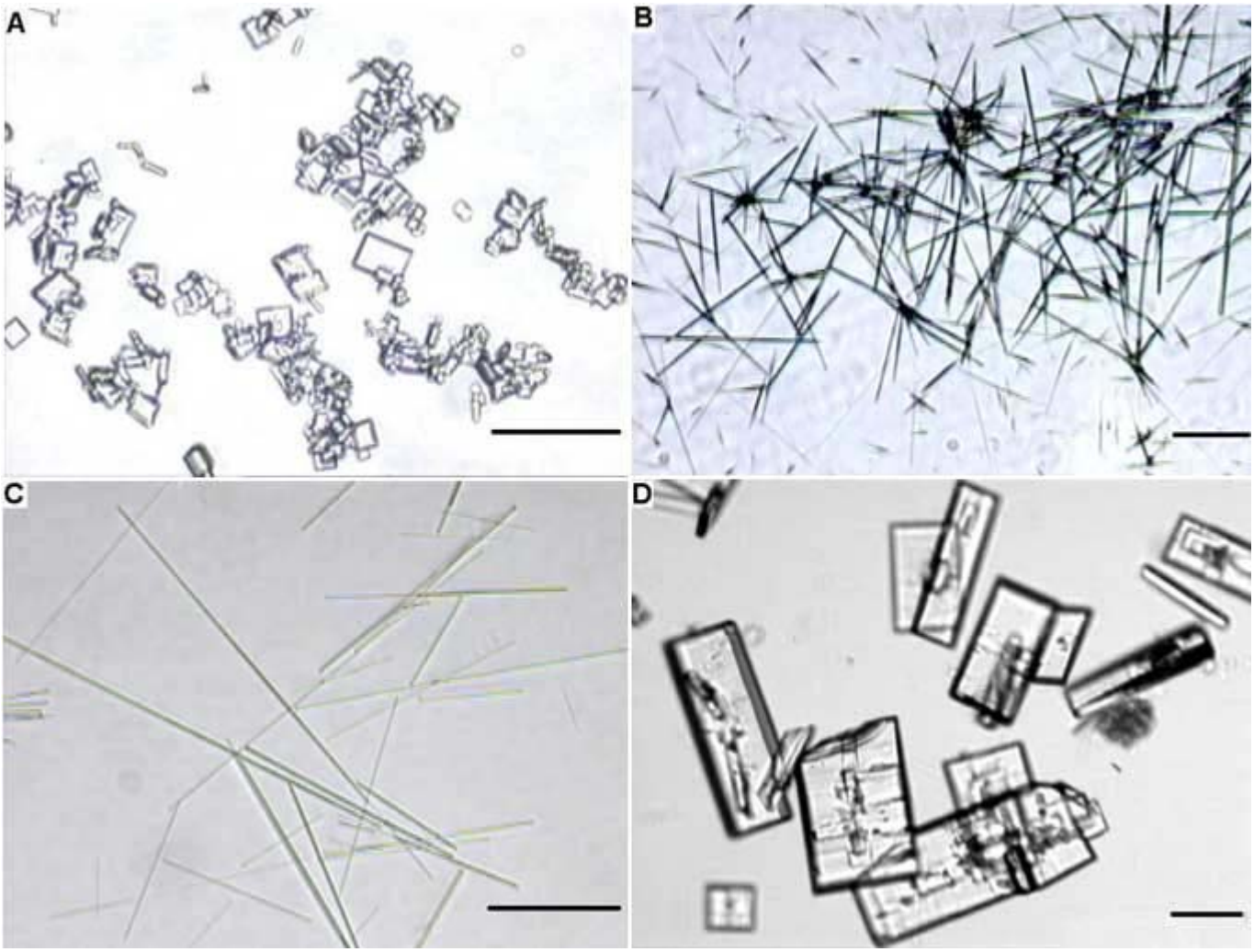

Figure 6. Micrographs of the crystals: (A) Em·2H_2O (bar 50 μm), (B) Em·MeEtCO, (C) Em·*i*-PrOH and (D) Em·EtOH (bar 100 μm).

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